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APPLICATION NOTE

Label-free assessment of B cell differentiation

INTRODUCTION

B cells are critical for a functioning immune system. Differentiated plasma B cells help the body defend against foreign entities through the production of antibodies. This antigen-specific antibody production by B-cells has also been exploited for the development of targeted therapeutics for cancer and immune disorders. In addition to playing a protective role in the adaptive immune system, dysfunctional B cells can also lead to life-threatening immunological disorders. Due to the importance of B cells for human health, studying the process of B-cell activation and differentiation into plasma cells is an important part of life science research. However, highly-specific surface markers for identifying plasma B cells are lacking and impose a challenge for researchers who want to isolate, characterize, and use them for research. In this proof-of-concept study, we used ghost cytometry to generate label-free markers of B cell differentiation into plasma cells. The approach described here has potential clinical applications for cell line development and the generation of B cell therapies.

RESULTS

Human B cells were cultured for 6 days under conditions that promoted either B cell activation (i.e., IgM and CD40 stimulation) or plasma cell differentiation (i.e., IgM, CD40, IL021, CpG ODN stimulation). IgD, CD38, and CD27 were used as 'ground truth' markers to define populations of B cells (defined as CD38(-)) and plasma cells (defined as IgD(-) / CD38high / CD27high). Ghost cytometry was used to generate ghost motion image (GMI) signals for each population. Using the GMI signals from the gated populations, a classifier was built using supervised machine learning. The GMI-based classifier showed excellent classification of B cells and plasma cells with an AUC score of 0.941 (**Figure 1**).

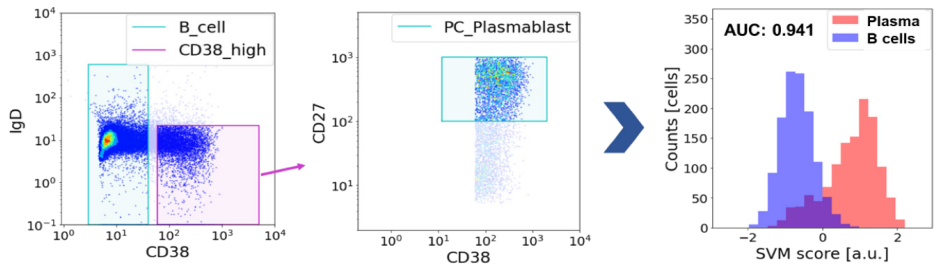

Figure 1. Development and assessment of the GMI-based classifiers for B-cell subtypes. B cells were defined as CD38 (-) and plasma cells were defined as IgD(-) / CD38^{high} / CD27^{high} using ground truth labels. A machine learning classifier was built from the GMI signals of the gated populations using ghost cytometry and achieved excellent performance in discerning B cells from plasma cells with an AUC score of 0.941.

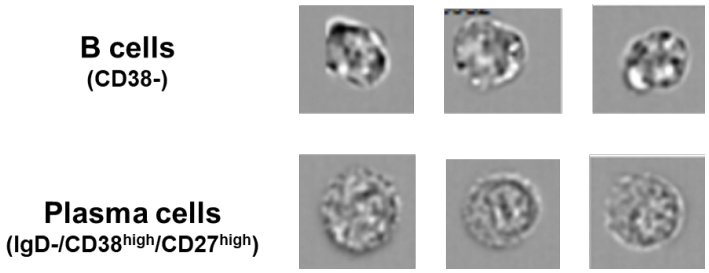

Figure 2. Distinct morphological differences, reflective of changes during the activation / plasma cell differentiation process, were observed in microscopic images of the different B-cell populations (taken with an Amnis Flowsight instrument at 60X magnification).

SUMMARY

Ghost cytometry can identify and classify activated B cells and differentiated plasma cells without the use of external molecular markers or labels. This has practical applications for research programs where isolation of B cell subsets without external stimulation is needed to preserve cells for further downstream R&D applications.

THINKCYTE INC.

UNITED STATES
733 Industrial Road
San Carlos, CA 94070

JAPAN
7-3-1 Hongo,
Bunkyo, Tokyo

CONTACT@THINKCYTE.COM WWW.THINKCYTE.COM